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Hormonal response of Arctic fox females to short- and long-term stress

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ABSTRACT: The aim of this study was to determine the extent of the hormonal response of Arctic fox females exposed to two types of stress: short-term and long-term, combined with adaptation to new environmental conditions. Short-term stress (20 min) was investigated in 34 females on farm A in the Wielkopolska province. The testing procedure involved capturing of the animals, their immobilisation, phenotype evaluation, and placing in target cages. Blood for laboratory examinations was collected from the *vena cephalica antebrachii* three times: on the day of the test, directly after the procedure and after three days. Long-term stress (8 h), combined with adaptation to new environmental conditions, was examined in 30 females which were purchased from a farm in the Lodzkie province (farm B). The testing procedure involved selection and capture of the animals, immobilisation and transfer from the pavilion, blood collection and placing of animals in a transport cage. Transport of the animals to the target farm (farm A) lasted approximately 8 h. Blood was collected five times in total, i.e. before transport (on farm B), after the transport to farm A, and then after three days, whereas the last two samplings were conducted at a 5- and 15-day interval from the third blood collection. The control group consisted of 20 randomly selected females from farm A. Blood from these animals was collected twice – at the beginning and at the end of the experiment. Changes in hormone concentrations (cortisol and testosterone) were adopted as a measure of sensitivity to a stressor. Blood hormones were analysed using a radio-immunological method. The course of stress reaction was affected by exposure time and stressor intensity, and significant changes in cortisol ($P \leq 0.01$) and testosterone ($P \leq 0.05$) concentrations were noted among individuals subjected to both short-term and long-term stress. Increased cortisol concentrations were maintained for a longer time in the group of Arctic fox females exposed to a long-term stress. In conclusion, the course of a stress reaction is affected by the duration of exposure and intensity of the stressor, and the strong stress reaction to zootechnical treatments and transport confirms the lack of complete domestication of this species.

Keywords: cortisol; testosterone; stress; transport

Moderate stress profitably affects immunity since it contributes to adaptation processes, whereas an excessive secretion of glucocorticoids indicates an adverse effect involving reduced mucus secretion by stomach and duodenal mucosa, decalcification, reduced efficiency of the immune system, increased risk of cardiovascular diseases, as well as reproduction disorders and reduced productivity (Kania et al. 1999; Broom 2006). Unprofitable factors resulting in changes in stress hormone concentrations include transport, changes in the surroundings and handling personnel, repeated immobilisation of the animals, veterinary and breeding procedures and also brutal treatment of the animals. The disturbance of natural homeostasis, depending on the intensity of the stressor and degree of stress compensation possible, may be a signal triggering adaptation mechanisms to a new environment,

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which is manifested in a behavioural reaction combined with an increased synthesis of hormones of the hypo-physical-adrenal axis (Kolacz and Bodak 1999; Morrow et al. 2002; Mostl and Palme 2002; Aoyama et al. 2003; Breuer et al. 2003; Wierzbicki et al. 2005; Nowakowicz-Debek et al. 2006). One of the methods to evaluate stress and its intensity is the analysis of hormones involved in stress and adaptation reactions. The most frequently examined hormones include: catecholamines (adrenalin, noradrenalin), adrenocorticotropic hormone, progesterone and testosterone, vasopressin and aldosterone, and corticoids, i.e. cortisol or corticosterone, depending on the animal species (Jakubowski et al. 1993; Kania et al. 1999; Osadchuk et al. 2001; Wierzbicki et al. 2005). Stress occurrence may be also determined by analysis of packed cell volume, leukocyte count, ketone bodies or enzyme evaluation (lactate dehydrogenase and keratin phosphokinase), whereas evaluation of the acid-base balance is a useful tool in determining the stress response (Becerril-Herrera et al. 2010; Roldan-Santiago et al. 2011a; Roldan-Santiago et al. 2011b; Trujillo-Ortega et al. 2011; Mota-Rojas et al. 2011, Mota-Rojas et al. 2012a; Mota-Rojas et al. 2012b).

Among many hormones like catecholamines (adrenalin, noradrenalin), adrenocorticotropic hormone, progesterone and testosterone, vasopressin and aldosterone, cortisol or corticosterone, cortisol and testosterone are most often selected for examination in fur animals undergoing stress (Moe and Bakken 1996; Kania et al. 1999; Osadchuk et al. 2001; Wierzbicki et al. 2005).

The aim of this study was to determine the extent of hormonal reaction of Arctic fox females subjected to two types of stress: short-term and long-term, combined with an adaptation to new environmental conditions, by the analysis of changes in blood cortisol and testosterone concentrations.

MATERIAL AND METHODS

We examined six-month-old Arctic fox females kept on a farm located in the western part of the Wielkopolska province (farm A), and Arctic fox females purchased from a farm from the Lodzkie province (farm B). The females from farm A were exposed to short-term stress, while females from farm B were subjected to long-term stress, combined with adaptation to new environmental conditions on farm A.

The short-term stress (20 min) was investigated in 34 females selected randomly from 110 animals previously introduced to the basic herd for the subsequent breeding season on farm A. The females were subjected to manipulations lasting 20 min. The testing procedure involved capture of the animals placed in two-row pavilions (using neck tongs), their immobilisation and movement from the pavilion to the examination table where they were subjected to phenotype evaluation. After evaluation, the females were transferred to individual cages (200 × 100 × 80 cm), in a part of the farm separated from the rest of the animals (change of the cage type and its localisation). Blood for laboratory analysis was collected three times: on the testing day (before the test and directly after the end of the procedure), as well as after three days.

Long-term stress, combined with adaptation to new environmental conditions, was examined in 30 females (one-year old), purchased from the farm in the Lodzkie province (farm B). The testing procedure involved selection and capture of the animals placed in two-row pavilions (using neck tongs), their immobilisation and transfer from the pavilion, blood collection and the placement of each animal in a transport cage, which was then loaded onto a truck. The transport of purchased animals to the target farm (farm A) lasted approximately 8 h. After transfer to farm A where the experiment was conducted, the animals were individually placed in cages (200 × 100 × 80 cm). Blood from the animals was collected five times in total, i.e. before transport (on the original farm), directly after the transfer to the new farm, and after three days, while the last two samplings were conducted at 5- and 15-day intervals from the third blood collection.

The control group consisted of 20 randomly selected one-year-old females, originating from farm A, not intended for basic herd replacement. Blood from these animals was collected twice – at the beginning and at the end of the experiment.

Changes in hormone concentrations (cortisol and testosterone) were adopted in the experiment as a measure of sensitivity to a stressor. The hormones were examined in blood serum using the radio-immunological method (Ruder et al. 1972; Demetriou 1987) with reagents from DPC Biermann. Blood samples in a volume of 1–2 ml were collected on the farms from the *vena cephalica antebrachii,* within a timeframe not exceeding 3 min from the moment of animal capture. The samples were always collected

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at the same time in order to exclude circadiurnal fluctuations in hormone concentrations. During the course of biological material collection, the animals were not in contact with one another (they were placed in cages distant from the basic herd).

The study was conducted with the agreement of the 2nd Local Ethical Committee of Wroclaw University of Environmental and Life Sciences.

Statistical analysis was conducted using SAS 9.1.3 computer software. GLM (General Linear Models) was used to evaluate the influence of particular effects on the analysed hormones. Analysis of variance was conducted using a constant linear model containing group and sample number effect for an evaluation of the changes in the examined hormones (the data were subject to logarithmic transformation). The significance of differences between the means was evaluated using the Duncan test. Relationships between concentrations of both hormones in five subsequent samplings were determined using straight Pearson's correlation.

RESULTS

The type of stress factor and time of exposure of animals resulted in significant differences in the hormonal response of the organism, as measured by cortisol levels in animal blood, (Table 1).

The animals exposed to stress lasting a few hours (transport, new farm) demonstrated significantly lower ($P \leq 0.01$) cortisol levels in blood serum compared to individuals which were subjected to intense discomfort-inducing treatments in short time intervals (catching, immobilisation, cage change). Increased cortisol concentrations were maintained for a significantly longer time period in the group of foxes which were exposed to the long-term stress.

The twenty-minute manipulation, including routine zootechnical and veterinary practices, caused a strong neuroendocrine system response (Table 2).

Changes in stress hormone secretion were also observed among the females, which were transported from one farm to another and were incorporated into the new herd (Table 3).

The lowest levels of cortisol and testosterone ($P \leq 0.01$) were noted before application of the stressor, in the first collection – still on the home farm. The eight-hour transport and accompanying activities caused the strongest reaction; cortisol levels increased nearly four-fold, and testosterone levels by about 72%.

At the 3rd sampling, the cortisol concentrations were still high and similar to the level noted three days earlier (Table 3). A significant decrease in cortisol levels ($P \leq 0.01$) was noted at the 4th and 5th samplings, and was accompanied by a systematic increase in testosterone in blood serum. In the third week of the adaptation process, cortisol concentrations approached the physiological values but testosterone secretion was still two-fold higher.

Non-significant fluctuations in cortisol and testosterone were noted in control animals (Table 4) selected from the basic herd, and were probably caused by the blood collection procedure (Kowalski et al. 1996). Experimental females, compared to the controls, were characterised by significantly higher cortisol levels in the initial and final phases of the experiment, and significantly higher ($P \leq 0.05$) testosterone concentrations at the last sampling (Table 4). The main reason for the differences in the levels of adrenal cortex hormones was the change

Table 1. Changes in cortisol and testosterone concentrations depending upon exposure time and type of stressor

	Sampling number	Exposure time			
		short-term (n = 34)		long-term (n = 30)	
		$\bar{x}$	sd	$\bar{x}$	sd
Cortisol (nmol/l)	1	53.94	12.27	49.48	15.45
	2	298.99^{A}	64.14	194.66^{B}	60.67
	3	131.39^{a}	63.41	171.99^{b}	67.67
Testosterone (nmol/l)	1	0.48	0.24	0.36	0.13
	2	0.61	0.61	0.62	0.41
	3	0.44	0.44	0.59	0.28

a,b = differences in rows significant at $P \leq 0.05$

A,B = differences in rows significant at $P \leq 0.01$

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Table 2. Changes in cortisol and testosterone concentrations during short-term stress

Sampling number	*n*	Cortisol (nmol/l)		Testosterone (nmol/l)	
		$\bar{x}$	sd	$\bar{x}$	sd
1	34	53.94^{A}	12.27	0.48^{a}	0.24
2	33	298.99^{B}	64.14	0.61^{Ab}	0.61
3	34	131.39^{C}	63.41	0.44^{B}	0.44

a,b = differences in rows significant at $P \leq 0.05$

A,B,C = differences in rows significant at $P \leq 0.01$

Table 3. Changes in cortisol and testosterone concentrations during long-time stress

Sampling number	*n*	Cortisol (nmol/l)		Testosterone (nmol/l)	
		$\bar{x}$	sd	$\bar{x}$	sd
1	26	49.48^{A}	15.45	0.36^{A}	0.13
2	29	194.66^{B}	60.67	0.62^{B}	0.41
3	29	171.99^{B}	67.67	0.59^{B}	0.28
4	30	88.38^{C}	37.12	0.63^{B}	0.26
5	30	78.14^{AC}	30.96	0.69^{B}	0.43

A,B,C = differences in columns significant at $P \leq 0.01$

in the function of the endocrine system which accompanied the adaptation process.

Pearson's simple correlation (Table 5) between the level of examined hormones obtained in the analysed period was –0.18 ($P \leq 0.03$). There were significant, positive relationships between the level of the same hormone in successive samplings (Table 5), and also negative (average and high) correlation indices between the level of cortisol and testosterone in successive samplings.

DISCUSSION

A stress reaction elicits mobilisation of the sympathetic-suprarenal system, and depending on the strength of the stimulus, also mobilisation of the pituitary-cortex-suprarenal axis. Capture, immobilisation and transport of animals related to place change as well as practices related to blood sampling belong to the same group of affection-psychological stressors activating the neuroendocrine axis.

Immobilisation of pigs for blood collection caused short-term fluctuations in cortisol levels (Kowalski et al. 1996). An increase in cortisol concentrations during a short-term transport of pigs was described by Averos et al. (2007), which then fell again after their unloading in the slaughter house. In turn, Tsuchiya and Horii (1995), after an initial increase, noted a decrease in testosterone levels in the blood serum of Syrian hamsters as a result of 30-min immobilisation. Systematic increases

Table 4. Changes in stress hormone concentrations in the blood serum of females at the beginning and end of the adaptation process

	Period	Experimental group (n = 30)		Control group (n = 20)	
		$\bar{x}$	sd	$\bar{x}$	sd
Cortisol (nmol/l)	I	194.66^{A}	60.67	61.98^{B}	14.93
	II	78.14^{a}	30.96	53.29^{b}	12.30
Testosterone (nmol/l)	I	0.62	0.41	0.58	0.29
	II	0.69^{a}	0.43	0.51^{b}	0.22

a,b = differences in rows significant at $P \leq 0.05$

A,B = differences in rows significant at $P \leq 0.01$

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Table 5. Pearson's correlation coefficients between cortisol (K1–K5) and testosterone (T1–T5) concentrations in the blood serum of arctic foxes in successive stages of the experiment

Hormone	T1	K2	T2	K3	T3	K4	T4	K5	T5
K1	–0.60***	0.69***	–0.45**	0.63***	–0.55**	0.74***	–0.45**	0.74***	–0.39
T1		–0.54**	0.50**	–0.54**	0.35	–0.47	0.04	–0.40	–0.01
K2			–0.66***	0.87***	–0.54**	0.54**	–0.50**	0.56**	–0.24
T2				–0.46*	0.73***	–0.20	0.36	–0.14	0.09
K3					–0.40*	0.71***	–0.42*	0.67***	–0.27
T3						–0.35	0.71***	–0.36	0.52**
K4							–0.24	0.88***	–0.19
T4								–0.42*	0.86***
K5									–0.42*

*correlation values significant at $P \leq 0.05$; **correlation values significant at $P \leq 0.01$; ***correlation values significant at $P \leq 0.001$

in cortisol during five successive blood samplings, with concurrent enhanced testosterone secretion, was noted by Moe and Bakken (1996) in a group of red foxes not subjected previously to a particular stressor. Breuer et al. (2003), in studies of the stress response, noted a higher increase in cortisol levels in the blood of heifers subjected to short-term manipulations, depending on the stimulus type and exposure time. A decrease in cortisol levels in the days following exposure of pigs to a chronic stress was observed by Jakubowski et al. (1993). Also, research on adaptive processes of Arctic foxes demonstrated a decrease in the concentration of this hormone and an increase in testosterone levels in successive samplings after cessation of increased cortisol secretion (Wierzbicki et al. 2005). Similarly, an increased secretion of testosterone in the course of the chronic stress was reported by Tsuchiya and Horii (1995).

In our study, testosterone levels increased at the 2nd blood sampling (after transport of animals to the new farm) in both groups and then decreased at the 3rd sampling (i.e. three days later) but this tendency was not confirmed statistically.

Barnett and Hemsworth (after Kolacz and Bodak 1999) examined the reaction of animals to the presence of humans and emotional stress, and noted high fluctuations in the levels of corticosteroids, which ranged from –4% to +64% of the initial concentration. According to different authors, the reasons for the disturbances in the hormonal balance of the suprarenal cortex include changes in the environment, inappropriate handling of the animals, veterinary practices and even feeding (Bakken 1998; Breuer et al. 2003; Wierzbicki et al. 2005; Nowakowicz-Debek et al. 2006).

Kowalski (1998) focused particularly on the increase in cortisol concentrations in a group of animals subjected to the stress of immobilisation. Situations resulting from everyday routine work on the farm can lead to apprehension and fear, which consequently elicit an increased synthesis of stress hormones. According to Filistowicz et al. (1999), the reaction of animals to routine husbandry and veterinary practices may be improved by reducing unpleasant situations to a minimum or by the selection of animals behaving calmly in the presence of adverse stimuli.

In our experiment, highly significant differences in cortisol levels were noted between subsequent blood samplings (Table 2). A greater than 5.5-fold increase in cortisol levels in blood serum was observed directly after cessation of the stress (2nd sampling) compared to the first sampling, and a significantly lower concentration of that hormone ($P \leq 0.01$) was found on the 3rd day (3rd sampling). Despite the fact that the animals were not disturbed during the two subsequent days, cortisol levels in the 3rd sample were significantly higher compared to the baseline level (Table 2). It is likely that the relatively short time intervals between blood samplings led the foxes to associate certain activities and the presence of the research team with expected discomfort. Also Moe and Bakken (1996) reported high cortisol levels in subsequent blood samplings in the case of red fox females.

Henry's model (Kowalski 1998) assumes the existence of two types of stress responses depending on the predisposition of the organism: hormonal

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(with increased cortisol secretion) and behavioural, relating to an intensified production of testosterone. Changes in testosterone concentrations in the blood of Syrian hamsters which were related to the duration of the stress were observed by Tsuchiya and Horii (1995) – the authors noted an increased testosterone secretion during animal immobilisation and then, after discomfort cessation, a decrease in the concentration of this hormone below the physiological level. Testosterone fluctuations were explained by the researchers by mutual relations of the pituitary-cortical-suprarenal system and hippocampus in the course of the stress response.

In our study, highly significant fluctuations in testosterone levels were noted concurrently with an increase in blood serum cortisol concentrations. The exposure of female foxes to stress caused about a 27% increase in "fight hormone" secretion. After stressor cessation, testosterone levels returned to values close to the initial level.

Transport, place change and also repeated capture and weighing are activities which are very stressful for non-domesticated animals. A high intensity of negative stimuli could have elicited strong fear, behavioural changes and disturbed physiological balance of the organism in the group of foxes, forcing the animals to mobilise adaptive reactions. Negative effects of long-term manipulation cause disturbances in the functioning of the psycho-neuroendocrine system, as confirmed in numerous studies (Jakubowski et al. 1993; Tsuchiya and Horii 1995; Moe and Bakken 1996; Grandin 1997; Anderson et al. 1999; Aoyama et al. 2003; Wierzbicki et al. 2005; Tadich et al. 2008).

A decrease in cortisol secretion during the course of the adaptation of Arctic foxes to new environmental conditions was reported by Wierzbicki et al. (2005) and Nowakowicz-Debek et al. (2006). Jakubowski et al. (1993), in the course of evaluating immune indices of pigs under chronic stress conditions, also noted a considerable decrease in cortisol over the course of the experiment. A study of the long-term stress response of Arctic foxes showed higher testosterone levels on subsequent days of adaptation, particularly among excitable and physically active individuals (Wierzbicki et al. 2005). Two- and six-hour immobilisation conducted on a population of Syrian hamsters also elicited an increase in "fight" hormone levels after an initial decrease (Tsuchiya and Horii 1995).

Improved adaptative abilities are observed among organisms less susceptible to stress, and are characterised by lower cortisol levels in blood serum. The adaptive ability of an organism depends to a high degree on genotype, age and sex of the animal, and also on previous experiences in stressful situations (Kostro et al. 2007).

Moderate and highly negative correlations between cortisol and testosterone levels in subsequent samplings were noted in our study, which may indicate mutual interactions of the pituitary – suprarenal cortex axis and the limbic system. Moreover, we found significant, positive correlations between the levels of these hormones in subsequent samplings.

The authors of a report concerning the adaptation of Arctic foxes observed opposing relations between cortisol and testosterone fluctuations. The regression coefficient of cortisol concentrations observed during the experiment reached –0.305 ng/ml, while for testosterone it was +0.01 l/ml per day (Wierzbicki et al. 2005).

An injection of 5α-dihydrotestosterone can cause a decrease in cortisol secretion in the blood serum of goats during the course of a negative emotional reaction, caused by transport (Aoyama et al. 2003). The study of Elman and Breier (1997) demonstrated an increase in cortisol concentrations, with a concurrent decrease in testosterone levels, in the blood serum of men subjected to metabolic stress. A decrease in the concentration of this hormone was also noted by Francis (1981) in a group of men subjected to strong emotional stress. In turn, Fenske (1996) observed a decrease in testosterone levels in the blood serum of guinea pigs after injection of the synthetic adrenocorticotropic hormone. A study conducted on rats (Frye and Edinger 2004) suggests that testosterone metabolism in the hippocampus can significantly regulate animal behaviour in threatening and also stressful situations. The more rapid response of the neuroendocrine axis of females in threatening situations (Hansen and Damgaard 1991; Anderson et al. 1999; Osadchuk et al. 2001; Osadchuk et al. 2003; Wierzbicki et al. 2005) may indicate a role for testosterone as a hormone which reduces emotional stimulation and attenuates symptoms of pain and fear (Aikey et al. 2002).

CONCLUSION

It may be concluded that the course of a stress reaction is affected by the duration of exposure and

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intensity of the stressor. In the animals exposed to short-term and long-term stress significant changes in the concentration of the analysed hormones were found. Arctic fox females exposed to long-term stress had elevated blood serum cortisol concentrations that persisted for a longer period of time. On the other hand, short-term exposure to a stressor did not cause full activation of the neuroendocrine system in foxes.

Pearson's simple correlations demonstrated significant relationships between cortisol and testosterone levels at the subsequent stages of the stress reaction and adaptation to the new environmental conditions. The existence of an opposing relationship between the examined hormones in subsequent samplings points to mutual interactions of the pituitary-cortical-adrenal axis and the limbic system. The strong stress reaction to zootechnical treatments and transport confirms the lack of complete domestication in the examined species.

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